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NOTE FOR GUIDANCE ON THE INVESTIGATION OF BIOAVAILABILITY AND BIOEQUIVALENCE

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1 INTRODUCTION

To exert an optimal therapeutic action an active moiety should be delivered to its site of action in an effective concentration for the desired period. To allow reliable prediction of the therapeutic effect the performance of the dosage form containing the active substance should be well characterised.

In the past, several therapeutic misadventures related to differences in bioavailability (e.g. digoxin, phenytoin, primidone) testify to the necessity of testing the performance of dosage forms in delivering the active substance to the systemic circulation and thereby to the site of action. Thus the bioavailability of an active substance from a pharmaceutical product should be known and reproducible. This is especially the case if one product containing one certain active substance is to be used instead of its innovator product. In that case the product should show the same therapeutic effect in the clinical situation. It is generally cumbersome to assess this by clinical studies.

Comparison of therapeutic performances of two medicinal products containing the same active substance is a critical means of assessing the possibility of alternative use between the innovator and any essentially similar medicinal product. Assuming that in the same subject an essentially similar plasma concentration time course will result in essentially similar concentrations at the site of action and thus in an essentially similar effect, pharmacokinetic data instead of therapeutic results may be used to establish equivalence: bioequivalence.

It is the objective of this guidance to define, for products with a systemic effect, when bioavailability or bioequivalence studies are necessary and to formulate requirements for their design, conduct, and evaluation. The possibility of using *in vitro* instead of *in vivo* studies with pharmacokinetic end points is also envisaged.

This guideline should be read in conjunction with Directive 75-318/EEC, as amended, and other pertinent elements outlined in current and future EU and ICH guidelines and regulations especially those on:

- Pharmacokinetic Studies in Man
- Modified Release Oral and Transdermal Dosage Forms: Section I (Pharmacokinetic and Clinical Evaluation)
- Modified Release Oral and Transdermal Dosage Forms: Section II (Quality)
- Investigation of Chiral Active Substances.
- Fixed Combination Medicinal Products
- Clinical Requirements for Locally Applied, Locally Acting Products Containing Known Constituents.
- The Investigation of Drug Interactions
- Development Pharmaceutics
- Process Validation
- Manufacture of the Finished Dosage Form
- Validation of analytical procedures: Definitions and Terminology (ICH topic Q2A)
- Validation of analytical procedures: Methodology (ICH topic Q2B)
- Structure and Content of Clinical Study Reports (ICH topic E3)
- Good Clinical Practice: Consolidated Guideline (ICH topic E6)
- General Considerations for Clinical Trials (ICH topic E8)
- Statistical Principles for Clinical Trials (ICH topic E9)
- Choice of Control Group in Clinical Trials (ICH topic E10)
- Amendments to Commission Regulation on (EC) 542/95
- Common Technical Document (ICH topic M4)

For medicinal products not intended to be delivered into the general circulation the common

systemic bioavailability approach cannot be applied. Under these conditions the (local) availability may be assessed, where necessary, by measurements quantitatively reflecting the presence of the active substance at the site of action using methods specially chosen for that combination of active substance and localisation (see section 5.1.8). In this case, as well as in others, alternative methods may be required such as studies using pharmacodynamic end points. Furthermore, where specific requirements for different types of products are needed, the appropriate exceptions are mentioned therein.

This Note for Guidance does not explicitly apply to biological products.

2 DEFINITIONS

Before defining bioavailability and related terminology some definitions pertaining to dosage and chemical forms are given:

2.1 Pharmaceutical equivalence

Medicinal products are pharmaceutically equivalent if they contain the same amount of the same active substance(s) in the same dosage forms that meet the same or comparable standards.

Pharmaceutical equivalence does not necessarily imply bioequivalence as differences in the excipients and/or the manufacturing process can lead to faster or slower dissolution and/or absorption.

2.2 Pharmaceutical alternatives

Medicinal products are pharmaceutical alternatives if they contain the same active moiety but differ in chemical form (salt, ester, etc.) of that moiety or in the dosage form or strength.

2.3 Bioavailability

Bioavailability means the rate and extent to which the active substance or active moiety is absorbed from a pharmaceutical form and becomes available at the site of action.

In the majority of cases substances are intended to exhibit a systemic therapeutic effect, and a more practical definition can then be given, taking into consideration that the substance in the general circulation is in exchange with the substance at the site of action:

> -Bioavailability is understood to be the extent and the rate at which a substance or its active moiety is delivered from a pharmaceutical form and becomes available in the general circulation.

It may be useful to distinguish between the "absolute bioavailability" of a given dosage form as compared with that (100%) following intravenous administration (e.g. oral solution *vs.* iv.), and the "relative bioavailability" as compared with another form administered by the same or another non intravenous route (e.g. tablets vs. oral solution).

2.4 Bioequivalence

Two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same.

Alternatively to classical bioavailability studies using pharmacokinetic end points to assess bioequivalence, other types of studies can be envisaged, e.g. human studies with clinical or pharmacodynamic end points, studies using animal models or in vitro studies as long as they are appropriately justified and/or validated.

2.5 Essentially similar products

The current EU definition for essentially similar products is as follows (see "The rules governing medicinal products in the European Union", Notice to Applicants, Vol. 2A in accordance with the December 1998 European Court of Justice ruling in the "Generics" case):

"A medicinal product is essentially similar to an original product where it satisfies the criteria of having the same qualitative and quantitative composition in terms of active substances, of having the same pharmaceutical form, and of being bioequivalent unless it is apparent in the light of scientific knowledge that it differs from the original product as regards safety and efficacy".

By extension, it is generally considered that for immediate release products the concept of essential similarity also applies to different oral forms (tablets and capsules) with the same active substance.

The need for a comparative bioavailability study to demonstrate bioequivalence is identified under 5.1. Concerns about differences in essentially similar medicinal products lie on the use of different excipients and methods of manufacture that ultimately might have an influence on safety and efficacy. A bioequivalence study is the widely accepted means of demonstrating that these differences have no impact on the performance of the formulation with respect to rate and extent of absorption, in the case of immediate release dosage forms. It is desirable that excipients must be devoid of any effect or their safe use is ensured by appropriate warning in the package label – see guideline on excipients in the label and package leaflet: "The Rules Governing Medicinal Products in the European Union", 1998, Vol. 3B, - and not interfere with either the release or the absorption process.

An essentially similar product can be used instead of its innovator product. An 'innovator' product is a medicinal product authorised and marketed on the basis of a full dossier i.e. including chemical, biological, pharmaceutical, pharmacological-toxicological and clinical data. A 'Reference Product' must be an 'innovator' product (see 3.5).

2.6 Therapeutic equivalence

A medicinal product is therapeutically equivalent with another product if it contains the same active substance or therapeutic moiety and, clinically, shows the same efficacy and safety as that product, whose efficacy and safety has been established.

In practice, demonstration of bioequivalence is generally the most appropriate method of substantiating therapeutic equivalence between medicinal products, which are pharmaceutically equivalent or pharmaceutical alternatives, provided they contain excipients generally recognised as not having an influence on safety and efficacy and comply with labelling requirements with respect to excipients. (see 2.5).

However, in some cases where similar extent of absorption but different rates of absorption are observed the products can still be judged therapeutically equivalent if those differences are not of therapeutic relevance. A clinical study to prove that differences in absorption rate are not therapeutically relevant will probably be necessary.

3 DESIGN AND CONDUCT OF STUDIES

In the following sections, requirements for the design and conduct of bioavailability or bioequivalence studies are formulated. It is assumed that the applicant is familiar with pharmacokinetic theories underlying bioavailability studies. The design should be based on a reasonable knowledge of the pharmacodynamics and/or the pharmacokinetics of the active substance in question. For the pharmacokinetic basis of these studies reference is made to the recommendation "Pharmacokinetic studies in man". The design and conduct of the study

should follow EU-regulations on Good Clinical Practice, including reference to an Ethics Committee.

A bioequivalence study is basically a comparative bioavailability study designed to establish equivalence between test and reference products. The following sections apply mainly to bioequivalence studies. Since bioavailability studies are comparative in nature, the contents of the following sections apply to these studies as well, with the necessary adaptations in accordance with the aim of each specific study. Where necessary, specific guidance concerning bioavailability studies will be given.

The methodology of bioequivalence studies can be used to assess differences in the pharmacokinetic parameters in pharmacokinetic studies such as drug-drug or food–drug interactions or to assess differences in subsets of the population. In this case the relevant guidelines should be followed and the selection of subjects, the design and the statistical analysis should be adjusted accordingly.

3.1 Design

The study should be designed in such a way that the formulation effect can be distinguished from other effects. If the number of formulations to be compared is two, a two-period, two-sequence crossover design is often considered to be the design of choice.

However, under certain circumstances and provided the study design and the statistical analyses are scientifically sound alternative well-established designs could be considered such as parallel design for very long half-life substances and replicate designs for substances with highly variable disposition.

In general, single dose studies will suffice, but there are situations in which steady-state studies

- may be required, e.g. in the case of
  - dose- or time-dependent pharmacokinetics,
  - some modified release products (in addition to single dose investigations),
- or can be considered, e.g.
  - if problems of sensitivity preclude sufficiently precise plasma concentration measurements after single dose administration.
  - if the intra-individual variability in the plasma concentration or disposition precludes the possibility of demonstrating bioequivalence in a reasonably sized single dose study and this variability is reduced at steady state.

In such steady-state studies the administration scheme should follow the usual dosage recommendations.

The number of subjects required is determined by

a) the error variance associated with the primary characteristic to be studied as estimated from a pilot experiment, from previous studies or from published data,

b) the significance level desired,

c) the expected deviation from the reference product compatible with bioequivalence (delta) and

d) the required power.

The clinical and analytical standards imposed may also influence the statistically determined number of subjects. However, generally the minimum number of subjects should be not smaller than 12 unless justified.

Subsequent treatments should be separated by adequate wash out periods. In steady-state studies wash out of the previous treatment last dose can overlap with the build-up of the second treatment, provided the build-up period is sufficiently long (at least three times the terminal half-life).

The sampling schedule should be planned to provide an adequate estimation of C_{max} and to cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of absorption. This is generally achieved if the AUC derived from measurements is at least 80% of the AUC extrapolated to infinity. If a reliable estimate of terminal half-life is necessary, it should be obtained by collecting at least three to four samples during the terminal log linear phase.

In order to study bioavailability under steady-state conditions when differences between morning and evening or nightly dosing are known, (e.g. if it is known that the circadian rhythm is known to have an influence on bioavailability), sampling should be carried out over a full 24 hours cycle.

For drugs with a long half-life, relative bioavailability can be adequately estimated using truncated AUC as long as the total collection period is justified. In this case the sample collection time should be adequate to ensure comparison of the absorption process.

3.2 Subjects

3.2.1 Selection of subjects

The subject population for bioequivalence studies should be selected with the aim to minimise variability and permit detection of differences between pharmaceutical products. Therefore, the studies should normally be performed with healthy volunteers. The inclusion/exclusion criteria should be clearly stated in the protocol.

Subjects could belong to either sex; however, the risk to women of childbearing potential should be considered on an individual basis.

In general, subjects should be between 18 - 55 years old and of weight within the normal range according to accepted normal values for the Body Mass Index. They should be screened for suitability by means of clinical laboratory tests, an extensive review of medical history, and a comprehensive medical examination. Depending on the drug's therapeutic class and safety profile special medical investigations may have to be carried out before, during and after the completion of the study. Subjects should preferably be non-smokers and without a history of alcohol or drug abuse. If moderate smokers are included (less than 10 cigarettes per day) they should be identified as such and the consequences for the study results should be discussed.

3.2.2 Standardisation of the study

The test conditions should be standardised in order to minimise the variability of all factors involved except that of the products being tested. Therefore, standardisation of the diet, fluid intake and exercise is recommended. Subjects should preferably be fasting at least during the night prior to administration of the products. If the Summary of Product Characteristics of the reference product contains specific recommendations in relation with food intake related to food interaction effects the study should be designed accordingly.

The time of day for ingestion should be specified and as fluid intake may profoundly influence gastric passage for oral administration forms, the volume of fluid (at least 150 ml) should be constant. All meals and fluids taken after the treatment should also be standardised in regard to composition and time of administration during the sampling period. The subjects should not take other medicines during a suitable period before and during the study and should abstain from food and drinks, which may interact with circulatory, gastrointestinal,

liver or renal function (e.g. alcoholic or xanthine-containing beverages or certain fruit juices). As the bioavailability of an active moiety from a dosage form could be dependent upon gastrointestinal transit times and regional blood flows, posture and physical activity may need to be standardised.

3.2.3 Inclusion of patients

If the investigated active substance is known to have adverse effects and the pharmacological effects or risks are considered unacceptable for healthy volunteers it may be necessary to use patients instead, under suitable precautions and supervision. In this case the applicant should justify the alternative.

3.2.4 Genetic phenotyping

Phenotyping and/or genotyping of subjects should be considered for exploratory bioavailability studies and all studies using parallel group design. It may be considered as well in crossover studies (e.g. bioequivalence, dose proportionality, food interaction studies etc.) for safety or pharmacokinetic reasons. If a drug is known to be subject to major genetic polymorphism, studies could be performed in panels of subjects of known phenotype or genotype for the polymorphism in question.

3.3 Characteristics to be investigated

In most cases evaluation of bioavailability and bioequivalence will be based upon the measured concentrations of the parent compound. In some situations, however, measurements of an active or inactive metabolite may be necessary instead of the parent compound. Such situations include cases where the use of a metabolite may be advantageous to determine the extent of drug input, e.g. if the concentration of the active substance is too low to be accurately measured in the biological matrix (e.g. major difficulty in analytical method, product unstable in the biological matrix or half-life of the parent compound too short) thus giving rise to significant variability.

Bioequivalence determinations based on metabolites should be justified in each case bearing in mind that the aim of a bioequivalence study is intended to compare the *in vivo* performance of test and reference products. In particular if metabolites significantly contribute to the net activity of an active substance and the pharmacokinetic system is non-linear, it is necessary to measure both parent drug and active metabolite plasma concentrations and evaluate them separately.

In bioavailability studies, the shape of and the area under the plasma concentration *versus* time curves are mostly used to assess extent and rate of absorption. The use of urine excretion data may be advantageous in determining the extent of drug input in case of products predominately excreted renally, but has to be justified when used to estimate the rate of absorption. Sampling points or periods should be chosen, such that the time- concentration profile is adequately defined so as to allow the estimation of relevant parameters.

From the primary results, the bioavailability characteristics desired are estimated, namely AUC_t, AUC_∞, C_{max}, t_{max}, Ae_t, Ae_∞ as appropriate, or any other justifiable characteristics (cf. Appendix I). The method of estimating AUC-values should be specified. For additional information $t_{1/2}$ and MRT can be estimated. For studies in steady state AUC_τ, C_{max}, C_{min} and fluctuation should be provided.

In bioequivalence studies the AUC_t is the most reliable reflection of the extent of absorption.

The exclusive use of compartmental based estimates are not recommended.

If pharmacodynamic effects are used as characteristics the measurements should provide a sufficiently detailed time course, the initial values in each period should be comparable and the complete effect curve should remain below the maximum physiological response.

Specificity, accuracy and reproducibility of the methods should be sufficient. The non-linear character of the dose/response relationship should be taken into account and base line corrections should be considered during data analysis.

3.4 Chemical analysis

The bioanalytical part of bioequivalence trials should be conducted according to the applicable principles of Good Laboratory Practice (GLP).

The bioanalytical methods used to determine the active moiety and/or its biotransformation product(s) in plasma, serum, blood or urine or any other suitable matrix must be well characterised, fully validated and documented to yield reliable results that can be satisfactorily interpreted. The main objective of method validation is to demonstrate the reliability of a particular method for the quantitative determination of an analyte(s) concentration in a specific biological matrix. The characteristics of a bioanalytical method essential to ensure the acceptability of the performance and the reliability of analytical results are: (1) stability of the stock solutions and of the analyte(s) in the biological matrix under processing conditions and during the entire period of storage; (2) specificity; (3) accuracy; (4) precision (5) limit of quantification and (6) response function.

The validation of a bioanalytical method should comprise two distinct phases: (1) the pre-study phase in which the compliance of the assay with the six characteristics listed above is verified and (2) the study phase itself in which the validated bioanalytical method is applied to the actual analysis of samples from the biostudy mainly in order to confirm the stability, accuracy and precision.

A calibration curve should be generated for each analyte in each analytical run and it should be used to calculate the concentration of the analyte in the unknown samples in the run. A number of separately prepared Quality Control samples should be analysed with processed test samples at intervals based on the total number of samples. In addition, it is necessary to validate the method of processing and handling the biological samples.

All procedures should be performed according to pre-established Standard Operating Procedures (SOPs). All relevant procedures and formulae used to validate the bioanalytical method should be submitted and discussed. Any modification of the bioanalytical method before and during analysis of study specimens may require adequate revalidation; all modifications should be reported and the scope of revalidation justified.

According to the requirements of the note for guidance on the "Investigation of Chiral Active Substances", bioequivalence studies supporting applications for essentially similar medicinal products containing chiral active substances should be based upon enantiomeric bio-analytical methods unless (1) both products contain the same stable single enantiomer; (2) both products contain the racemate and both enantiomers show linear pharmacokinetics.

3.5 Reference and test product

Test products in an application for a generic product are normally compared with the corresponding dosage form of an innovator (see 2.5) medicinal product (reference product). The choice of reference product should be justified by the applicant.

For an abridged application claiming essential similarity to a reference product, application to numerous Member States based on bioequivalence with a reference product from one Member State can be made.

Such an application can be considered acceptable unless there is a significant difference between the reference products originating from the same manufacturer (or its subsidiaries/licensees), in terms of the qualitative and quantitative composition in excipients. Concerned Member States may request information from the first Member State on the

reference product, namely on the composition, manufacturing process and finished product specification.

Where additional bioequivalence studies are required, they should be carried out using the product registered in the concerned Member State as the reference product

It should be remembered that the development of the test product should always take into account the Note for Guidance on "Development Pharmaceutics".

The test products used in the biostudy must be prepared in accordance with GMP-regulations. Batch control results of the test product should be reported.

In the case of oral solid forms for systemic action the test product should usually originate from a batch of at least 1/10 of production scale or 100 000 units, whichever is greater, unless otherwise justified. The production of batches used should provide a high level of assurance that the product and process will be feasible on an industrial scale; in case of production batch smaller than 100 000 units, a full production batch will be required. If the product is subjected to further scale-up this should be properly validated.

Samples of the product from full production batches should be compared with those of the test batch, and should show similar in vitro dissolution profiles when employing suitable dissolution test conditions (see Appendix II).

The study sponsor will have to retain a sufficient number of all investigational product samples in the study for one year in excess of the accepted shelf life or two years after completion of the trial or until approval whichever is longer to allow re-testing, if it is requested by the authorities.

In accordance with Annex 13 to the EU guide to GMP, reference and test product must be packed in an individual way for each subject included in the bioequivalence trial. Every effort should be made to allow a precise tracking of administration of the reference and test products to the subjects, for instance by the use of labels with a tear-off portion.

3.6 Data analysis

The primary concern of bioequivalence assessment is to quantify the difference in bioavailability between the reference and test products and to demonstrate that any clinically important difference is unlikely.

3.6.1 Statistical analysis

The statistical method for testing relative bioavailability (e.g. bioequivalence) is based upon the 90% confidence interval for the ratio of the population means (Test/Reference), for the parameters under consideration.

This method is equivalent to the corresponding two one-sided test procedure with the null hypothesis of bioinequivalence at the 5% significance level. The statistical analysis (e.g. ANOVA) should take into account sources of variation that can be reasonably assumed to have an effect on the response variable. A statistically significant sequence effect should be handled appropriately.

Pharmacokinetic parameters derived from measures of concentration, e.g. AUC, C_{max} should be analysed using ANOVA. The data should be transformed prior to analysis using a logarithmic transformation.

If appropiate to the evaluation the analysis technique for t_{max} should be non-parametric and should be applied to untransformed data. For all pharmacokinetic parameters of interest in addition to the appropriate 90% confidence intervals for the comparison of the two formulations, summary statistics such as median, minimum and maximum should be given.

3.6.2 Acceptance range for pharmacokinetic parameters

The pharmacokinetic parameters to be tested, the procedure for testing and the acceptance ranges should be stated beforehand in the protocol.

In studies to determine average bioequivalence the acceptance intervals for the main characteristics are detailed as follows:

AUC-ratio

The 90% confidence interval for this measure of relative bioavailability should lie within an acceptance interval of 0.80-1.25. In specific cases of a narrow therapeutic range the acceptance interval may need to be tightened.

In rare cases a wider acceptance range may be acceptable if it is based on sound clinical justification.

C_{max}-ratio

The 90% confidence interval for this measure of relative bioavailability should lie within an acceptance interval of 0.80-1.25. In specific cases of a narrow therapeutic range the acceptance interval may need to be tightened.

In certain cases a wider interval may be acceptable. The interval must be prospectively defined e.g. 0.75-1.33 and justified addressing in particular any safety or efficacy concerns for patients switched between formulations.

Others

Statistical evaluation of t_{max} only makes sense if there is a clinically relevant claim for rapid release or action or signs related to adverse effects. The non-parametric 90% confidence interval for this measure of relative bioavailability should lie within a clinically determined range.

For other (see 3.3) pharmacokinetic parameters in comparison relative bioavailability (e.g. C_{min}, Fluctuation, $t_{1/2}$, etc.) considerations analogous to those for AUC, C_{max} or t_{max} apply, taking into consideration the use of log-transformed or untransformed data, respectively.

3.6.3 Handling deviations from the study plan

The method of analysis should be planned in the protocol. The protocol should also specify methods for handling drop-outs and for identifying biologically implausible outliers. Post hoc exclusion of outliers is generally not accepted. If modelling assumptions made in the protocol (e.g. for extrapolating AUC to infinity) turn out to be invalid, a revised analysis in addition to the planned analysis (if this is feasible) should be presented and discussed.

3.6.4 A remark on individual and population bioequivalence

To date, most bioequivalence studies are designed to evaluate average bioequivalence. Experience with population and individual bioequivalence studies is limited. Therefore, no specific recommendation is given on this matter.

3.7 In vitro dissolution complementary to a bioequivalence study

The results of "in vitro" dissolution tests, obtained with the batches of test and reference products that were used in the bioequivalence study should be reported. The results should be reported as profiles of percent of labelled amount dissolved versus time.

The specifications for the *in vitro* dissolution of the product should be derived from the dissolution profile of the batch that was found to be bioequivalent to the reference product and would be expected to be similar to those of the reference product (see Appendix II).

For immediate release products, if the dissolution profile of the test product is dissimilar

compared to that of the reference product and the in vivo data remain acceptable the dissolution test method should be re-evaluated and optimised. In case that no discriminatory test method can be developed which reflects in vivo bioequivalence a different dissolution specification for the test product could be set.

3.8 Reporting of results

The report of a bioavailability or a bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with GCP-rules and related EU and ICH E3 guidelines. This implies that the authenticity of the whole of the report is attested by the signature of the principal investigator. The responsible investigator(s), if any, should sign for their respective sections of the report.

Names and affiliations of the responsible investigator (s), site of the study and period of its execution should be stated. The names and batch numbers of the products used in the study as well as the composition(s), finished product specifications and comparative dissolution profiles should be provided. In addition, the applicant should submit a signed statement confirming that the test product is the same as the one that is submitted for marketing authorisation.

All results should be clearly presented and should include data from subjects who eventually dropped-out. Drop-out and withdrawal of subjects should be fully documented and accounted for. The method used to derive the pharmacokinetic parameters from the raw data should be specified. The data used to estimate AUC should be reported. If pharmacokinetic models are used to evaluate the parameters the model and computing procedure used should be justified. Deletion of data should be justified.

All individual subject data should be given and individual plasma concentration/time curves presented in linear/linear and log/linear scale. The analytical report should include the results for all standard and quality control samples as well. A representative number of chromatograms or other raw data should be included covering the whole concentration range for all, standard and quality control samples as well as the specimens analysed. The analytical validation report should be submitted as well.

The statistical report should be sufficiently detailed to enable the statistical analysis to be repeated, e.g. randomisation scheme, demographic data, values of pharmacokinetic parameters for each subject, descriptive statistics for each formulation and period. A detailed ANOVA and/or non-parametric analysis, the point estimates and corresponding confidence intervals including the method of their estimation should also be included.

4 APPLICATIONS FOR PRODUCTS CONTAINING NEW ACTIVE SUBSTANCES

4.1 Bioavailability

In the case of new active substances (new chemical entities) intended for systemic action, the pharmacokinetic characterisation will have to include the determination of the systemic availability of the substance in its intended pharmaceutical form in comparison with intravenous administration. If this is not possible (e.g. not technically feasible or for safety reasons) the bioavailability relative to a suitable oral solution or suspension should be determined. In the case of a prodrug the intravenous reference solution should preferably be made of the active moiety.

4.2 Bioequivalence

During development bioequivalence studies are necessary as bridging studies between (i) pivotal and early clinical trial formulations; (ii) pivotal clinical trial formulations, especially those used in the dose finding studies, and the to-be-marketed medicinal product; (iii) other

comparisons depending on the situation. Such studies may be exempted if the absence of differences in the in vivo performance can be justified by satisfactory in vitro data (see 5.1.1 and 5.2).

5 APPLICATIONS FOR PRODUCTS CONTAINING APPROVED ACTIVE SUBSTANCES

5.1 Bioequivalence studies

In vivo bioequivalence studies are needed when there is a risk that possible differences in bioavailability may result in therapeutic inequivalence.

The kind of studies to be performed may vary with the type of product, as follows.

5.1.1 Oral Immediate Release Forms with Systemic Action

This section pertains to dosage forms such as tablets, capsules and oral suspensions and takes into consideration criteria derived from the concepts underlying the Biopharmaceutics Classification System, i.e. high solubility, high permeability for the active substance and high dissolution rate for the medicinal product. These criteria, along with a non-critical therapeutic range should be primarily considered; therefore the following characteristics have to be taken into account in order to justify the request for exemption from in vivo bioequivalence studies. Hence data must be supplied to justify the absence of such studies.

a) Characteristics related to the active substance:

i - risk of therapeutic failure or adverse drug reactions:

this risk depends on the requirements of special precautions with respect to precision and accuracy of dosing of the active substance, e.g. the need for critical plasma concentrations;

ii - risk of bioinequivalence:

evidence of bioavailability problems or bioinequivalence exists for some specific active substances;

iii – solubility:

When the active substance is highly water soluble, the product could be in general exempted from bioquivalence studies unless, considering the other characteristics, the exemption could entail a potential risk. Polymorphism and particle size are major determinants of dissolution rate and special attention should be paid to these characteristics. An active substance is considered highly water soluble if the amount contained in the highest dose strength of an immediate release product is dissolved in 250 ml of each of three buffers within the range of pH 1-8 at 37°C (preferably at or about pH 1.0, 4.6, 6.8);

iv - pharmacokinetic properties:

linear and complete absorption indicating high permeability reduces the possibility of an immediate release dosage form influencing the bioavailability.

b) Characteristics related to the medicinal product:

i - rapid dissolution

in case of exemption from bioequivalence studies, in vitro data should demonstrate the similarity of dissolution profile between the test product and the reference product in each of three buffers within the range of pH 1-8 at 37°C (preferably at or about pH 1.0, 4.6, 6.8). However, in cases where more than 85% of the active substance are dissolved within 15 minutes, the similarity of dissolution

profiles may be accepted as demonstrated (see appendix II);

ii – excipients

the excipients included in the composition of the medicinal product are well established and no interaction with the pharmacokinetics of the active substance is expected. In case of atypically large amounts of known excipients or new excipients being used, additional documentation has to be submitted;

iii – manufacture

the method of manufacture of the finished product in relation with critical physicochemical properties of the active substance (e.g. particle size, polymorphism) should be adequately addressed and documented in the development pharmaceutics section of the dossier.

5.1.2 Oral solutions

If the product is an aqueous oral solution at time of administration and contains an active substance in the same concentration as an oral solution currently approved as a medicinal product, no bioequivalence study is required, provided the excipients contained in it do not affect gastrointestinal transit, absorption or in vivo stability of the active substance.

In those cases where an oral solution has to be tested against an oral immediate release formulation a comparative bioavailability study will be required unless an exemption can be justified (see 5. 1. 1).

5.1.3 Non-Oral Immediate Release forms with systemic action

In general bioequivalence studies are required.

5.1.4 Modified Release and transdermal dosage forms

Requirements for bioequivalence studies in accordance with the specific guideline

5.1.5 Fixed combinations products

Combination products should in general be assessed with respect to bioavailability and bioequivalence of individual active substances either separately (in the case of a new combination) or as an existing combination. Criteria under 5.1.1 will apply to individual components. The study in case of a new combination should be designed in such a way that the possibility of a pharmacokinetic drug-drug interaction could be detected.

5.1.6 Parenteral solutions

The applicant is not required to submit a bioequivalence study if the product is to be administered as an aqueous intravenous solution containing the same active substance in the same concentration as the currently authorised product.

In the case of other parenteral routes, e.g. intramuscular or subcutaneous, if the product is of the same type of solution (aqueous or oily), contains the same concentration of the same active substance and the same or comparable excipients as the medicinal product currently approved, then bioequivalence testing is not required.

5.1.7 Gases

If the product is a gas for inhalation a bioequivalence study is not required.

5.1.8 Locally applied products

a) Locally acting

For products for local use (after oral, nasal, inhalation, ocular, dermal, rectal, vaginal etc. administration) intended to act without systemic absorption the approach to determine

bioequivalence based on systemic measurements is not applicable and pharmacodynamic or comparative clinical studies are in principle required. The lack of them should be justified (see specific Note for Guidance).

Whenever systemic exposure resulting from locally applied, locally acting medicinal products entails a risk of systemic adverse reactions, systemic exposure should be measured.

b) Systemically acting

For locally applied products with systemic action a bioequivalence study is always required.

5.2 In Vitro Dissolution

Dissolution studies are always necessary and consequently required. . In vitro dissolution testing forms a part of the assessment of a bioequivalence waiver request based on criteria as described in section 5.1. Dissolution studies must follow the guidance as laid out in Appendix II.

5.3 Variations

If a product has been reformulated from the formulation initially approved or the manufacturing method has been modified by the manufacturer in ways that could be considered to impact on the bioavailability, a bioequivalence study is required, unless otherwise justified. Any justification presented should be based upon general considerations, e.g. as per 5.1.1, or on whether an acceptable in vivo / in vitro correlation has been established.

In cases where the bioavailability of the product undergoing change has been investigated and an acceptable correlation between in vivo performance and in vitro dissolution has been established, the requirements for in vivo demonstration of bioequivalence can be waived if the dissolution rate in vitro of the new product is similar to that of the already approved medicinal product under the same test conditions as used to establish the correlation (see Appendix II)

In all other cases bioequivalence studies have to be performed.

For variations of the innovator product the reference product for use in bioequivalence and dissolution studies is usually that authorised under the current formula, manufacturing method, packaging etc. and the product manufactured in line with the proposed changes is tested against this.

When variations to an essentially similar product are made the reference product for the bioequivalence study should be the innovator product.

5.4 Dose proportionality in immediate release oral dosage forms

If a new application concerns several strengths of the active substance a bioequivalence study investigating only one strength may be acceptable. However the choice of the strength used should be justified on analytical, pharmacokinetic and safety grounds. Furthermore all of the following conditions should be fulfilled:

- the pharmaceutical products are manufactured by the same manufacturer and process;
- the drug input has been shown to be linear over the therapeutic dose range (if this is not the case the strengths where the sensitivity is largest to identify differences in the two products should be used);
- the qualitative composition of the different strengths is the same;
- the ratio between amounts of active substance and excipients is the same, or, in the case of preparations containing a low concentration of the active substance (less than 5%), the ratio between the amounts of excipients is similar;

- the dissolution profile should be similar under identical conditions for the additional strengths and the strength of the batch used in the bioequivalence study.

If a new strength (within the approved dose range) is applied for on the basis of an already approved medicinal product and all of the stated conditions hold then a bioequivalence study is not necessary.

5.5 Suprabioavailability

If suprabioavailability is found, i.e. if the new product displays an extent of absorption appreciably larger than the approved product, reformulation to a lower dosage strength should be considered. In this case, the biopharmaceutical development should be reported and a final comparative bioavailability study of the reformulated new product with the old approved product should be submitted.

In case reformulation is not carried out the dosage recommendations for the suprabioavailable product will have to be supported by clinical studies. Such a pharmaceutical product should not be accepted as therapeutically equivalent to the existing reference product. If marketing authorisation is obtained, the new product may be considered as a new medicinal product.

To avoid confusion for both prescribers and patients, it is recommended that the name of suprabioavailable product precludes confusion with the older approved product

Suprabioavailable products cannot claim "essential similarity" (see section 2.5) with the innovator product.

APPENDIX I

Explanation of the symbols in paragraph 3.3

C_{max}:	maximal plasma concentration;
C_{min}:	minimal plasma concentration;
C_{av}:	average plasma concentration;
t_{max}:	time passed since administration at which the plasma concentration maximum occurs;
AUC_t:	area under the plasma concentration curve from administration to last observed concentration at time t.
AUC_∞:	area under the plasma concentration curve extrapolated to infinite time;
AUC_τ:	AUC during a dosage interval in steady state;
MRT:	mean residence time;
Ae_t:	cumulative urinary excretion from administration until time t;
Ae_∞:	cumulative urinary excretion extrapolated to infinite time;
$t_{1/2}$:	plasma concentration half-life;
Fluctuation:	$(C_{max} - C_{min})/C_{av}$
Swing:	$(C_{max} - C_{min})/C_{min}$

APPENDIX II

Dissolution testing

A medicinal product is composed of drug substance and excipients and the proportion between them, the type of excipients and the manufacturing method of the final product are chosen based on the content, the physicochemical and the bulk properties of the drug and on its absorption properties. Taken as a whole this gives each product certain dissolution characteristics.

During the development of a medicinal product a dissolution test is used as a tool to identify formulation factors that are influencing and may have a crucial effect on the bioavailability of the drug. As soon as the composition and the manufacturing process are defined a dissolution test is used in the quality control of scale-up and of production batches to ensure both batch-to-batch consistency and that the dissolution profiles remain similar to those of pivotal clinical trial batches. Furthermore, a dissolution test can be used to support the bioavailability of a new drug product, the bioequivalence of an essentially similar product or variations.

Therefore, dissolution studies can serve several purposes:

i - Quality assurance

- To get information on the test batches used in bioavailability/bioequivalence studies and pivotal clinical studies to support specifications for quality control.
- To be used as a tool in quality control to demonstrate consistency in manufacture
- To get information on the reference product used in bioavailability/bioequivalence studies and pivotal clinical studies

ii -Bioequivalence surrogate inference

- To demonstrate similarity between reference products from different Member States
- To demonstrate similarity between different formulations of an active substance (variations and new, essentially similar products included) and the reference medicinal product
- To collect information on batch to batch consistency of the products (test and reference) to be used as basis for the selection of appropriate batches for the in vivo study.

The test methodology should be in accordance with pharmacopoeial requirements unless those requirements are shown to be unsatisfactory. Alternative methods can be considered when justified that these are discriminatory and able to differentiate between batches with acceptable and non-acceptable performance of the product in vivo.

If an active substance is considered highly soluble, it is reasonable to expect that it will not cause any bioavailability problems if, in addition, the dosage system is rapidly dissolved in the physiological pH-interval expected after product administration. A bioequivalence study may in those situations be waived based on case history and similarity of dissolution profiles which are based on discriminatory testing, provided that the other exemption criteria in 5.1.1 are met. The similarity should be justified by dissolution profiles, covering at least three time points, attained at three different buffers (normally pH range 1-6.8; in cases where it is considered necessary pH range 1-8).

In the case of a drug or excipients that are insensitive to pH, profiles from only two buffer systems are required.

If an active substance is considered to have a low solubility and a high permeability, the rate limiting step for absorption may be dosage form dissolution. This is also the case when one or more of the excipients are controlling the release and subsequent dissolution step of the active

substance. In those cases a variety of test conditions is recommended and adequate sampling should be performed until either 90% of the drug is dissolved or an asymptote is reached. Knowledge of dissolution properties under different conditions e.g. pH, agitation, ionic strength, surfactants, viscosity, osmotic pressure is important since the behaviour of the solid system in vivo may be critical for the drug dissolution independent of the physico-chemical properties of the active substance. An appropriate experimental statistical design may be used to investigate the critical parameters and for the optimisation of such conditions.

Any methods to prove similarity of dissolution profiles are accepted as long as they are justified.

The similarity may be compared by model-independent or model-dependent methods e.g. by linear regression of the percentage dissolved at specified time points, by statistical comparison of the parameters of the Weibull function or by calculating a similarity factor e.g the one defined below:

$$f_2 = 50 \bullet \log \left\{ \frac{100}{\sqrt{1 + \frac{\sum_{t=1}^{t=n} [R(t) - T(t)]^2}{n}}} \right\}$$

In this equation f_2 is the similarity factor, n is the number of time points, R (t) is the mean percent drug dissolved of e.g. a reference product, and T(t) is the mean percent drug dissolved of e.g. a test product.

The evaluation of similarity is based on the conditions of

- A minimum of three time points (zero excluded)
- 12 individual values for every time point for each formulation
- not more than one mean value of > 85% dissolved for each formulation
- that the standard deviation of the mean of any product should be less than 10% from second to last time point.

An f_2 value between 50 and 100 suggests that the two dissolution profiles are similar. In cases where more than 85% of the drug is dissolved within 15 minutes, dissolution profiles may be accepted as similar without further mathematical evaluation.

Guidance for Industry

BIOEQUIVALENCE GUIDANCE

(This version of the guidance replaces the version titled "Bioequivalence Guidance" that was made available on October 9, 2002. This document has been revised to update the contact information and typographical errors in the equation)

Section III.A. of this guidance has been superceded by CDER's guidance entitled "Bioanalytical Method Validation". Any general questions regarding the application of the Bioanalytical Method Validation guidance to new animal drugs should be directed to Marilyn Martinez, Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Pl., Rockville, MD 20855, (301)827-7577, marilyn.martinez@fda.hhs.gov. Any questions regarding analytical methods for tissue residues should be directed to Valerie Reeves, 7500 Standish Pl., Rockville, MD 20855, (301)827-6973, valerie.reeves@fda.hhs.gov.

This document is intended to provide guidance for the design and analysis of in vivo bioequivalence studies. This revision to the version that was made available in April 1996 adds an illustrative example of how to calculate confidence bounds when log transformed data are used.

Comments and suggestions regarding this guidance document should be submitted to the Divison of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the Docket Number (94D-0401). Additional information on the 1996 guidance document can be found in the Federal Register (Vol. 61, No. 102, May 24, 1996). Comments will be accepted at any time.

For questions regarding this guidance document, contact Ken J. Harshman, Center for Veterinary Medicine (HFV-104), Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine (CVM)
November 8, 2006

This guidance document represents the agency's current thinking on this matter. It does not create or confer any rights for or on any person and does not operate to bind the FDA or public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations or both.

PREAMBLE

In 1996, the Center for Veterinary Medicine (CVM) revised a document entitled "April 1990 Bioequivalence Guideline." The revised document, "Bioequivalence Guidance (Final) 1996", was issued in final form following notice and comment.

Many of the changes in the "Bioequivalence Guidance (Final) 1996" were based upon reports from panel presentations at the 1993 Veterinary Drug Bioequivalence Workshop in Rockville, Maryland, sponsored by the Center for Veterinary Medicine (CVM), the Animal Health Institute (AHI), the American Academy of Veterinary Pharmacology and Therapeutics (AAVPT), and the Animal Drug Alliance[1]. Some new topics were introduced into the guidance as a result of issues identified in the review of bioequivalence protocols and studies.

The major new topics in the guidance were as follows:

1. Higher than approved dose bioequivalence studies.
2. Bioequivalence testing for multiple strength solid oral dosage forms.
3. Assay considerations for bioequivalence studies.
4. AUC and CMAX as the pivotal parameters for bioequivalence determination.
5. Blood level bioequivalence studies to be accompanied by tissue residue depletion studies for generic products for food-producing animals.

CVM has revised the "1996 Bioequivalence Guidance" to add an illustrative example of how to calculate confidence bounds when log transformed data are used. The guidance has also been revised in accordance with FDA's Good Guidance Practices (GGPs, found in the Federal Register of February 27, 1997, 62 FR 8961). With the exception of the addition of information on how to calculate confidence bounds when log transformed data are used, minor revisions made to comply with the GGPs (e.g., addition of a cover sheet), and revisions to the Preamble,

the document is the same as the document issued in 1996. In September 2000, FDA revised the guidance to clarify sources of information more clearly.

A person may follow the guidance or may choose to follow alternate procedures or practices. If a person chooses to use alternate procedures or practices, that person may wish to discuss the matter further with the agency to prevent an expenditure of money and effort on activities that may later be determined to be unacceptable to FDA. Although this guidance document does not bind the agency or the public, and it does not create or confer any rights, privileges, or benefits for or on any person, it represents FDA's current thinking on bioequivalence testing for animal drugs. When a guidance document states a requirement imposed by statute or regulation, the requirement is law and its force and effect are not changed in any way by virtue of its inclusion in the guidance.

TABLE OF CONTENTS

I. INTRODUCTION

This document is intended to provide guidance for the design and analysis of *in vivo* bioequivalence studies. The guidance is an update of the April 12, 1990 Bioequivalence Guideline. Many of the changes in the guideline are based upon reports from panel presentations at the 1993 Veterinary Drug Bioequivalence Workshop[1].

Two products are considered to be bioequivalent when they are equally bioavailable; that is, equal in the rate and extent to which the active ingredient(s) or therapeutic ingredient(s) is (are) absorbed and become(s) available at the site(s) of drug action.

The Generic Animal Drug and Patent Term Restoration Act (GADPTRA) signed into law on November 16, 1988, permits sponsors to submit an Abbreviated New Animal Drug Application (ANADA) for a generic version of any off-patent approved animal drug (with certain exceptions noted in the law) regardless of whether the drug was approved prior to 1962 and subject to the National Academy of Sciences / National Research Council / Drug Effectiveness Study Implementation (NAS/NRC/DESI) review.

Bioequivalence studies are used in a variety of situations, most often when a sponsor proposes manufacturing a generic version of an approved off-patent product. A bioequivalence study may also be part of a new animal drug application (NADA) or supplemental NADA for approval of an alternative dosage form, new route of administration, or a significant manufacturing change which may affect drug bioavailability.

The Center has concluded that the tissue residue depletion of the generic product is not adequately addressed through bioequivalence studies. Therefore, sponsors of ANADA's for drug products for food-producing animals will generally be asked to include bioequivalence and tissue residue studies (21 USC 360 b (n) (1) (E)). A tissue residue study should generally accompany clinical end-point and pharmacologic end-point bioequivalence studies, and blood level bioequivalence studies that can not quantify the concentration of the drug in blood throughout the established withdrawal period (21 USC 360 b (n) (1) (A) (ii)).

Bioequivalence studies (*i.e.*, blood level, pharmacologic end-point, and clinical end-point studies) and tissue residue depletion studies should be conducted in accordance with good laboratory practice (GLP) regulations (21 CFR Part 58).

Whereas the focus of the guidance is bioequivalence testing for ANADA approval, the general principles also apply to relative bioavailability studies conducted for NADA's.

Sponsors should consult with the Center early in the product development process to facilitate the design of studies adequate for drug approval. The Center urges sponsors to submit protocols for review prior to conducting studies.

II. GENERAL CONSIDERATIONS

A. Selection of Reference Product for Bioequivalence Testing

As a general rule, the proposed generic product should be tested against the original pioneer product.

If the original pioneer product is no longer marketed, but remains eligible to be copied, then the first approved and available generic copy of the pioneer should be used as the reference product for bioequivalence testing against the proposed new generic product.

If several approved NADA's exist for the same drug product, and each approved product is labeled differently (*i.e.*, different species and/or claims), then the generic sponsor must clearly identify which product label is the intended pioneer. Bioequivalence testing should be conducted against the single approved product which bears the labeling that the generic sponsor intends to copy.

The generic sponsor should consult with CVM regarding selection of the appropriate reference product before conducting the bioequivalence study.

B. Criteria for Waiver of *In Vivo* Bioequivalence Study

The requirement for the in vivo bioequivalence study may be waived for certain generic products (21 USC 360 b (n) (1) (E)). Categories of products which may be eligible for waivers include, but are not limited to, the following:

1. Parenteral solutions intended for injection by the intravenous, subcutaneous, or intramuscular routes of administration.
2. Oral solutions or other solubilized forms.
3. Topically applied solutions intended for local therapeutic effects. Other topically applied dosage forms intended for local therapeutic effects for non-food animals only.
4. Inhalant volatile anesthetic solutions.

In general, the generic product being considered for a waiver contains the same active and inactive ingredients in the same dosage form and concentration and has the same pH and physico-chemical characteristics as an approved pioneer product.

However, the Center will consider bioequivalence waivers for non-food animal topical products with certain differences in the inactive ingredients of the pioneer and generic products.

If a waiver of the *in vivo* bioequivalence and/or the tissue residue study/studies is granted for a food animal drug product, then the withdrawal period established for the pioneer product will be assigned to the generic product.

Sponsors may apply for waivers of *in vivo* bioequivalence studies prior to submission of the ANADA's.

C. Selection of Blood Level, Pharmacologic End-point, or Clinical End-point Study

In vivo bioequivalence may be determined by one of several direct or indirect methods. Selection of the method depends upon the purpose of the study, the analytical method available, and the nature of the drug product. Bioequivalence testing should be conducted using the most appropriate method available for the specific use of the product.

The preferred hierarchy of bioequivalence studies (in descending order of sensitivity) is the blood level study, pharmacologic end-point study, and clinical end-point study. When absorption of the drug is sufficient to measure drug concentration *directly* in the blood (or other appropriate biological fluids or tissues) and systemic absorption is relevant to the drug action, then a blood (or other biological fluid or tissue) level bioequivalence study should be conducted. The blood level study is generally preferred above all others as the most sensitive measure of bioequivalence. The sponsor should provide justification for choosing either a pharmacologic or clinical end-point study over a blood-level (or other biological fluids or tissues) study.

When the measurement of the rate and extent of absorption of the drug in biological fluids can not be achieved or is unrelated to drug action, a pharmacologic end-point (*i.e.*, drug induced physiologic change which is related to the approved indications for use) study may be conducted. Lastly, in order of preference, if drug concentrations in blood (or fluids or tissues) are not measurable or are inappropriate, and there are no appropriate pharmacologic effects that can be monitored, then a clinical end-point study may be conducted, comparing the test (generic) product to the reference (pioneer) product and a placebo (or negative) control.

D. Species Selection

A bioequivalence study generally should be conducted for each species for which the pioneer product is approved on the label, with the exception of "minor" species (as defined in section 514.1 (d) (1) of Title 21 of the Code of Federal Regulations) on the label.

E. Dose Selection

Dose selection will depend upon the label claims, consideration of assay sensitivity, and relevance to the practical use conditions of the reference product. A blood level bioequivalence study should generally be conducted at the highest dose approved for the pioneer product.

However, the Center will consider a bioequivalence study conducted at a higher than approved dose in certain cases. Such a study may be appropriate when a multiple of the highest approved dose achieves measurable blood levels, but the highest approved dose does not. In general, the study would be limited to 2-3x the highest dose approved for the pioneer product. The pioneer product should have an adequate margin of safety at the higher than approved dose level. The generic sponsor should also confirm (*e.g.*, through literature) that the drug follows linear kinetics. A higher than approved dose bioequivalence study in food animal species would be accompanied by a tissue residue withdrawal study conducted at the highest approved dose for the

pioneer product.

For products labeled for multiple claims involving different pharmacologic actions at a broad dose range (*e.g.,* therapeutic and production claims), a single bioequivalence study at the highest approved dose will usually be adequate. However, multiple bioequivalence studies at different doses may be needed if the drug is known to follow nonlinear kinetics. The sponsor should consult with CVM to discuss the bioequivalence study or studies appropriate to a particular drug.

F. Multiple Strengths of Solid Oral Dosage Forms

The generic sponsor should discuss with CVM the appropriate *in vivo* bioequivalence testing and *in vitro* dissolution testing to obtain approval for multiple strengths (or concentrations) of solid oral dosage forms.

CVM will consider the ratio of active to inactive ingredients and the *in vitro* dissolution profiles of the different strengths, the water solubility of the drug, and the range of strengths for which approval is sought.

One *in vivo* bioequivalence study with highest strength product may suffice if the multiple strength products have the same ratio of active to inactive ingredients and are otherwise identical in formulation.

In vitro dissolution testing should be conducted, using an FDA approved method, to compare each strength of the generic product to the corresponding strength of the reference product.

G. Manufacturing of Pilot Batch ("Biobatch")

A pilot batch or "biobatch" should be the source of the finished drug product used in the pivotal studies (*i.e.*, bioequivalence studies and tissue residue studies), stability studies and the validation studies for the proposed analytical and stability indicating methods (refer to CVM's guidance number 42, "Animal Drug Manufacturing Guidelines").

III. BLOOD LEVEL STUDIES

Blood level bioequivalence studies compare a test (generic) product to a reference (pioneer) product using parameters derived from the concentrations of the drug moiety and/or its metabolites, as a function of time, in whole blood, plasma, serum (or in other appropriate biological fluids or tissues). This approach is particularly applicable to dosage forms intended to deliver the active drug ingredient(s) to the systemic circulation (*e.g.*, injectable drugs and most oral dosage forms). Generally, the study should encompass the absorption, distribution, and depletion (elimination) phases of the drug concentration *vs* time profiles.

A. Assay Consideration

A properly validated assay method is pivotal to the acceptability of any pharmacokinetic study. Sponsors should discuss any questions or problems concerning the analytical methodology with CVM before undertaking the bioequivalence studies. The ANADA submission should contain adequate information necessary for the CVM reviewer to determine the validity of the analytical

method used to quantitate the level of drug in the biological matrix (*e.g.*, blood).

The following aspects should be addressed in assessing method performance:

1. Concentration Range and Linearity

   The quantitative relationship between concentration and response should be adequately characterized over the entire range of expected sample concentrations. For linear relationships, a standard curve should be defined by at least 5 concentrations. If the concentration response function is non-linear, additional points would be necessary to define the non-linear portions of the curve. Extrapolation beyond a standard curve is not acceptable.

2. Limit of Detection (LOD)

   The standard deviation of the background signal and LOD should be determined. The LOD is estimated as the response value calculated by adding 3 times the standard deviation of the background response to the average background response.

3. Limit of Quantitation (LOQ)

   The initial determination of LOQ should involve the addition of 10 times the standard deviation of the background response to the average background response. The second step in determining LOQ is assessing the precision (reproducibility) and accuracy (recovery) of the method at the LOQ. The LOQ will generally be the lowest concentration on the standard curve that can be quantified with acceptable accuracy and precision (see items 5. and 6. below).

4. Specificity

   The absence of matrix interferences should be demonstrated by the analysis of 6 independent sources of control matrix. The effect of environmental, physiological, or procedural variables on the matrix should be assessed. Each independent control matrix will be used to produce a standard curve, which will be compared to a standard curve produced under chemically defined conditions. The comparison of curves should exhibit parallelism and superimposability within the limits of analytical variation established for the chemically defined standard curve.

5. Accuracy (Recovery)

   This parameter should be evaluated using at least 3 known concentrations of analyte freshly spiked in control matrix, one being at a point 2 standard deviations above the LOQ, one in the middle of the range of the standard curve ("mid-range") and one at a point 2 standard deviations below the upper quantitative limit of the standard curve. The accuracy of the method, based upon the mean value of 6 replicate injections, at each concentration level, should be within 80-120% of the nominal concentration at each level (high, mid-range, and LOQ).

6. Precision

   This parameter should be evaluated using at least 3 known concentrations of analyte freshly spiked in control matrix, at the same points used for determination of accuracy. The coefficient of variation (CV) of 6 replicates should be ± 10% for concentrations at or above 0.1 ppm (0.1 μ g/mL). A CV of ± 20% is acceptable for concentrations below 0.1 ppm.

7. Analyte Stability

   Stability of the analyte in the biological matrix under the conditions of the experiment (including any period for which samples are stored before analyses) should be established. It is recommended that the stability be determined with incurred analyte in the matrix of dosed animals in addition to, or instead of, control matrix spiked with pure analyte. Also, the influence of 3 freeze-thaw cycles at 2 concentrations should be determined.

   Stability samples at 3 concentrations should be stored with the study samples and analyzed through the period of time in which study samples are analyzed. These analyses will establish whether or not analyte levels have decreased during the time of analysis.

8. Analytical System Stability

   To assure that the analytical system remains stable over the time course of the assay, the reproducibility of the standard curve should be monitored during the assay. A minimal design would be to run analytical standards at the beginning and at the end of the analytical run.

9. Quality Control (QC) Samples

   The purpose of QC samples is to assure that the complete analytical method, sample preparation, extraction, clean-up, and instrumental analysis perform according to acceptable criteria. The stability of the drug in the text matrix for the QC samples should be known and any tendency for the drug to bind to tissue or serum components over time should also be known.

   Drug free control matrix, *e.g.*, tissue, serum, etc. that is freshly spiked known quantities of test drug, should be analyzed contemporaneously with test samples, evenly dispersed throughout each analytical run. This can be met by the determination of accuracy and precision of each analytical run (Items 5 and 6).

10. Replicate and Repeat Analyses

    Single rather than replicate analyses are recommended, unless the reproducibility and/or accuracy of the method are borderline. Criteria for repeat analyses should be determined prior to running the study and recorded in the method SOP.

11. Summary of Samples to Be Run With Each Analysis

a. Accuracy estimate (Item 5)
b. Precision estimate (Item 6)
c. Analytical system stability (Item 8)
d. Analyte stability samples (Item 7)

B. General Experimental Design Considerations

1. Dosing by Labeled Concentration

   The potency of the pioneer and generic products should be assayed prior to conducting the bioequivalence study to ensure that FDA or compendial specifications are met. The Center recommends that the potency of the pioneer and generic lots should differ by no more than ±5% for dosage form products.

   The animals should be dosed according to the labeled concentration or strength of the product, rather than the assayed potency of the individual batch (*i.e.*, the dose should not be corrected for the assayed potency of the product). The bioequivalence data or derived parameters should not be normalized to account for any potency differences between the pioneer and generic product lots.

2. Single Dose vs Multiple Dose Studies

   A single dose study at the highest approved dose will generally be adequate for the demonstration of bioequivalence. A single dose study at a higher than approved dose may be appropriate for certain drugs (refer to the section on Dose Selection).

   A multiple dose study may be appropriate when there are concerns regarding poorly predictable drug accumulation, (*e.g.* ,a drug with nonlinear kinetics) or a drug with a narrow therapeutic window. A multiple dose study may also be needed when assay sensitivity is inadequate to permit drug quantification out to 3 terminal elimination half-lives beyond the time when maximum blood concentrations (CMAX) are achieved, or in cases where prolonged or delayed absorption[2] exist. The determination of prolonged or delayed absorption (*i.e.*, flip-flop kinetics) may be made from pilot data, from the literature, or from information contained with FOI summaries pertaining to the particular drug or family of drugs.

3. Subject Characteristics

   Ordinarily, studies should be conducted with healthy animals representative of the species, class, gender, and physiological maturity for which the drug is approved. The bioequivalence study may be conducted with a single gender for which the pioneer product is approved, unless there is a known interaction of formulation with gender.

   An attempt should be made to restrict the weight of the test animals to a narrow range in order to maintain the same total dose across study subjects.

   The animals should not receive any medication prior to testing for a period of two weeks or more, depending upon the biological half-life of the ancillary drug.

4. Fed vs Fasted State

Feeding may either enhance or interfere with drug absorption, depending upon the characteristics of the drug and the formulation. Feeding may also increase the inter- and intrasubject variability in the rate and extent of drug absorption. The rationale for conducting each bioequivalence study under fasting or fed conditions should be provided in the protocol.

Fasting conditions, if used, should be fully described, giving careful consideration to the pharmacokinetics of the drug and the humane treatment of the test animals.

The protocol should describe the diet and feeding regime which will be used in the study.

If a pioneer product label indicates that the product is limited to administration either in the fed or fasted state, then the bioequivalence study should be conducted accordingly. If the bioequivalence study parameters pass the agreed upon confidence intervals, then the single study is acceptable as the basis for approval of the generic drug.

However, for certain product classifications or drug entities, such as enteric coated and oral sustained release products, demonstration of bioequivalence in both the fasted and the fed states may be necessary, if drug bioavailability is highly variable under feeding conditions, as determined from the literature or from pilot data. A bioequivalence study conducted under fasted conditions may be necessary to pass the confidence intervals. A second smaller study may be necessary to examine meal effects. CVM will evaluate the smaller study with respect to the means of the pivotal parameters (AUC, CMAX). The sponsors should consult with CVM prior to conducting the studies.

C. Pharmacokinetic and Statistical Considerations in Study Design

1. Sampling Time Considerations

The total number of sampling times necessary to characterize the blood level profiles will depend upon the curvature of the profiles and the magnitude of variability associated with the bioavailability data (including pharmacokinetic variability, assay error and interproduct differences in absorption kinetics).

The sampling times should adequately define peak concentration(s) and the extent of absorption. The sampling times should extend to at least 3 terminal elimination half-lives beyond TMAX. The sponsor should consult with CVM prior to conducting the pivotal bioequivalence study if the assay is unable to quantify samples to 3 half-lives.

Maximum sampling time efficiency may be achieved by conducting a pilot investigation. The pilot study should identify the general shapes of the test and reference curves, the magnitude of the difference in product profiles, and the noise associated with each blood sampling time (*e.g.*, variability attributable to assay error and the variability between subjects, for parallel study designs, or within subjects, for crossover study designs). This information should be applied to the determination of an optimum blood sampling

schedule. Depending upon these variability estimates, it may be more efficient to cluster several blood samples rather than to have samples which are periodically dispersed throughout the duration of blood sampling.[3]

2. Protein Binding

   In general, product bioequivalence should be based upon total (free plus protein bound) concentrations of the parent drug (or metabolite, when applicable). However, if nonlinear protein binding is known to occur within the therapeutic dosing range (as determined from literature or pilot data), then sponsors may need to submit data on both the free and total drug concentrations for the generic and pioneer products.

   Similarly, if the drug is known to enter blood erythrocytes, the protocol should address the issue of potential nonlinearity in erythrocyte uptake of the drug administered within the labeled therapeutic dosing range.

   The bioequivalence protocol or completed study report should provide any information available from the literature regarding erythrocyte uptake and protein binding characteristics of the drug or drug class, including the magnitude of protein binding and the type of blood protein to which it binds.

3. Subject Number

   Pilot studies are recommended as a means of estimating the appropriate sample size for the pivotal bioequivalence study. Estimated sample size will vary depending upon whether the data are analyzed on a log or linear scale. Useful references for sample size estimates include Westlake[4], Hauschke[5], and Steinijans[6].

4. Cross-over and Parallel Design Considerations

   A two-period cross-over design is commonly used in blood level studies. The use of cross-over designs eliminates a major source of study variability: between subject differences in the rates of drug absorption, drug clearance, and the volume of drug distribution.

   In a typical two-period cross-over design, subjects are randomly assigned to either sequence A or sequence B with the restriction that equal numbers of subjects are initially assigned to each sequence. The design is as follows:

	Sequence A	Sequence B
Period 1	Test	Reference
Period 2	Reference	Test

A crucial assumption in the two-period cross-over design is that of equal residual effects. Unequal residual effects may result, for example, from an inadequate washout period. Another assumption of the cross-over (or extended period) design is that there is no subject by formulation interaction. In other words, the assumption is that all subjects are from a relatively homogeneous population and will exhibit similar relative bioavailability of the test and reference products. If there are subpopulations of subjects, such that the relationship between product bioavailability is a function of the subpopulation within which they are being tested, then a subject by formulation interaction is said to exist.

A one-period parallel design may be preferable in the following situations:

a. The drug induces physiological changes in the animal (e.g., liver microsomal enzyme induction) which persist after total drug clearance and alter the bioavailability of the product administered in the second period.

b. The drug has a very long terminal elimination half-life, creating a risk of residual drug present in the animal at the time of the second period dosing.

c. The duration of the washout time for the two-period cross-over study is so long as to result in significant maturational changes in the study subjects.

d. The drug follows delayed or prolonged absorption (flip-flop kinetics[2]), where the slope of the [[beta]]-elimination phase is dictated by the rate of drug absorption rather than the rate of drug elimination from one or both products.

Other designs, such as the two-period design with four treatment sequences (Test/Test, Reference/Reference, Test/Reference and Reference/Test) or the extended period design may be appropriate depending on the circumstances. The use of alternative study designs should be discussed with CVM prior to conducting the bioequivalence study. Pilot data or literature may be used in support of alternative study designs.

5. Duration of Washout Time for Cross-over Study

For drugs which follow a one or two compartment open body model, the duration of the washout time should be approximately 10x the plasma apparent terminal elimination half-life, to provide for 99.9% of the administered dose to be eliminated from the body.

If more highly complex kinetic models are anticipated (*e.g.*, drugs for which long withdrawal times have been assigned due to prolonged tissue binding), or for drugs with the potential for physiologic carryover effects, the washout time should be adjusted accordingly.

The washout period should be sufficiently long to allow the second period of the cross-over study to be applicable in the statistical analysis. However, if sequence effects are noted, the data from the first period may be evaluated as a parallel design study.

6. Pivotal Parameters for Blood Level Bioequivalence

The sponsor is encouraged to calculate parameters using formulas which involve only the raw data (*i.e.*, so-called model independent methods).

a. Area Under the Curve (AUC) Estimates

The extent of product bioavailability is estimated by the area under the blood concentration *vs* time curve (AUC). AUC is most frequently estimated using the linear trapezoidal rule. Other methods for AUC estimation may be proposed by the sponsor and should be accompanied by appropriate literature references during protocol development.

For a single dose bioequivalence study, AUC should be calculated from time 0 (predose) to the last sampling time associated with quantifiable drug concentration AUC(0-LOQ). The comparison of the test and reference product value for this noninfinity estimate provides the closest approximation of the measure of uncertainty (variance) and the relative bioavailability estimate associated with AUC(0-INF), the full extent of product bioavailability[7].

The relative AUC values generally change very little once the absorption of both products has been completed[8,9]. However, because of the possibility of multifunctional absorption kinetics, it can not always be determined when the available drug has been completely absorbed. Therefore, CVM recommends extending the duration of sampling until such time that AUC(0-LOQ)/AUC(0-INF) > = 0.80. Generally, the sampling times should extend to at least 3 multiples of the drug's apparent terminal elimination half-life, beyond the time when maximum blood concentrations are achieved.

AUC(0-INF)should be used to demonstrate that the concentration-time curve can be quantitated such that AUC(0-LOQ)/AUC(0-INF) >= 0.80. The method for estimating the terminal elimination phase should be described in the protocol and the final study report. The AUC(0-LOQ)/AUC(0-INF) is calculated to determine whether AUC(0-LOQ) adequately reflects the extent of absorption.

The sponsor should consult with CVM if AUC(0-LOQ)/AUC(0-INF) is determined to be < 0.80. If AUC(0-LOQ)/AUC(0-INF) is <0.80, then a multiple dose study to steady state may be needed to allow an accurate assessment of AUC(0-INF) (where AUC(0-INF) = AUC(0-t) at steady state and t is the dosing interval).

In a multiple dose study, the AUC should be calculated over one complete dosing interval AUC(0-t). Under steady state conditions, AUC(0-t) equals the full extent of bioavailability of the individual dose AUC(0-INF) assuming linear kinetics. For drugs which are known to follow nonlinear kinetics, the sponsor should consult with CVM to determine the appropriate parameters for the bioequivalence determination.

b. Rate of Absorption

The rate of absorption will be estimated by the maximum observed drug concentration

(CMAX) and the corresponding time to reach this maximum concentration (TMAX).

When conducting a steady-state investigation, data on the minimum drug concentrations (trough values) observed during a single dosing interval (CMIN) should also be collected. Generally, three successive CMIN values should be provided to verify that steady-state conditions have been achieved. Although CMIN most frequently occurs immediately prior to the next successive dose, situations do occur with CMIN observed subsequent to dosing. To determine a steady state concentration, the CMIN values should be regressed over time and the resultant slope should be tested for its difference from zero.

c. Determination of Product Bioequivalence

Unless otherwise indicated by CVM during the protocol development for a given application, the pivotal bioequivalence parameters will be CMAX and AUC(0-LOQ) (for a single dose study) or AUC(0-t) (for a multiple dose study). To be indicative of product bioequivalence, the pivotal metrics should be associated with confidence intervals which fall within a set of acceptability limits (see Statistical Analysis section of this Guidance.

The sponsor and CVM should agree to the acceptable bounds for the confidence limits for the particular drug and formulation during protocol development.

If studies or literature demonstrate that the pioneer drug product exhibits highly variable kinetics, then the generic drug sponsor may propose alternatives to the generally acceptable bounds for the confidence limits.

TMAX in single dose studies and CMIN in multiple dose studies will be assessed by clinical judgment.

D. Statistical Analysis

CVM advocates the use of 90% confidence intervals, as the best available method for evaluating bioequivalence study data. Papers by Schuirmann[10] and Westlake[4] compare various methodologies for assessing drug product equivalence and describe the confidence interval approach.

The confidence interval approach should be applied to the individual parameters of interest (*e.g.*, AUC and CMAX). The sponsor may use untransformed or log transformed data. However, the choice of untransformed or log transformed data should be made by the sponsor with concurrence by the Center prior to conducting the study.

1. Untransformed Data

   A discussion of how the confidence interval approach should be applied to (normally distributed) untransformed data from a two-period crossover design is given below.

If we let $\overline{X}_{T1}$ be the mean for the test drug in period 1, $\overline{X}_{T2}$ the mean for the test drug in period 2, and $\overline{X}_{R1}$ and $\overline{X}_{R2}$ the respective means for the reference drug, then the estimates for the drugs averaged over both periods are $\overline{X}_T = (1/2)\left(\overline{X}_{T1} + \overline{X}_{T2}\right)$ for the test drug and $\overline{X}_R = (1/2)\left(\overline{X}_{R1} + \overline{X}_{R2}\right)$ for the reference drug. Although both sequence groups usually start with the same number of animals, the number of animals in each sequence group (n_A and n_B) that successfully finish the study may not be equal. The formulas above utilize the marginal or least squares estimates of μ_T and μ_R, the corresponding means in the target population. These means are not a function of the sample size in each sequence.

An analysis of variance is needed to obtain the estimate of σ^2, the error variance. The estimator, s^2, which will be used in the calculation of the 90% confidence interval should be obtained from the "error" mean square term found in the following ANOVA table.

Source	Degrees of Freedom
Sequence	1
Animal (Sequence)	n_A+n_B-2
Period	1
Formulation	1
Error	n_A+n_B-2
Total	$2n_A+2n_B-1$

Lower and upper 90% confidence intervals are then found by formulas based on Student's t-distribution.

$$L = \left(\overline{X}_T - \overline{X}_R\right) - t_{n_A+n_B-2;0.05}\, s \sqrt{\frac{1}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}$$

$$U = \left(\overline{X}_T - \overline{X}_R\right) + t_{n_A+n_B-2;0.05}\, s \sqrt{\frac{1}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}$$

The procedure of declaring two formulations bioequivalent if the 90% confidence interval is completely contained in some fixed interval, is statistically equivalent to performing two one-sided statistical tests ($\alpha = .05$) at the end-points of the interval.

Consider the following example with L = 3, U = 17, $\overline{X}_T$ = 110 and $\overline{X}_R$ = 100. By the traditional hypothesis testing approach, the result would be considered statistically significant since the confidence interval does not include 0. Using the confidence interval approach, the entire confidence interval lies within 17% of $\overline{X}_R$. (The lower end of the confidence interval lies within L/$\overline{X}_R$ = 3/100 = 3% of $\overline{X}_R$, while the upper end of the confidence interval lies within U/$\overline{X}_R$ = 17/100 = 17% of $\overline{X}_R$.) If it were determined by CVM that only differences larger than 20% were biomedically important, then using the confidence interval approach the results of this study would be considered adequate to demonstrate bioequivalence.

Now consider an example with L = -4, U = 24, $\overline{X}_T$ = 110 and $\overline{X}_R$ = 100. In this case, by the traditional hypothesis testing approach the result would not be considered statistically significant since the confidence interval includes 0. However, the confidence interval extends as far as 24% from $\overline{X}_R$. (The lower end of the confidence interval lies within L/$\overline{X}_R$ = -4/100 = -4% of $\overline{X}_R$, while the upper end of the confidence interval extends to U/$\overline{X}_R$ = 24/100 = 24% of $\overline{X}_R$.) If it were determined by CVM that only differences larger than 20% were biomedically important, then the results of this study would be considered inadequate to demonstrate bioequivalence, since the entire confidence interval is not within 20% of $\overline{X}_R$.

2. Logarithmically transformed data

This section discusses how the 90% confidence interval approach should be applied to log-transformed data. In this situation the individual animal AUC and CMAX values are log-transformed and the analysis is done on the transformed data. For a two-period crossover study, as described in D.1, the ANOVA model used to calculate estimates of the error variance and the least square means are identical for both transformed and untransformed data. The procedural difference comes after the lower and upper 90% confidence intervals are found by formulas based on Student's t-distribution.

The lower and upper confidence bounds of the log-transformed data will then need to be

back-transformed in order to be expressed on the original scale of the measurement. One thing to keep in mind when moving between the logarithm scale and the original scale is that the back-transformed mean of a set of data that has been transformed to the logarithm scale is not strictly equivalent to the mean that would be calculated from the data on the original scale of measurement. This back-transformed mean is known instead as the geometric mean.

It may help to see the calculations involved. If the AUC from each animal has been transformed to the logarithm scale, we can express the transformed AUC as *LnAUC*. Then the mean on the logarithm scale is as follows:

$\overline{LnAUC}_t = \sum_{i=1}^{n} \frac{LnAUC_{it}}{n}$ where the subscript *t* represents the AUC determinations for the test article, *i* is the AUC of the ith animal, and *n* is the total number of animals receiving the text article. When this mean is back-transformed, it becomes the geometric mean: $e^{(\overline{LnAUC}_t)}$. This geometric mean will be on the original scale of the measurement. It will be close to but not exactly equal to the mean obtained on the original scale of the measurement.

The back-transformation of the confidence bounds is accomplished in the following way:

Lower bound (expressed as a percentage)	=	$\left(e^{L}-1\right)\times 100$
Upper bound (expressed as a percentage)	=	$\left(e^{U}-1\right)\times 100$
Where	*L*	is the lower 90% confidence interval as given in Section III D 1 and calculated on the log-transformed data;
	U	is the upper 90% confidence interval as given in Section III D 1 and calculated on the log-transformed data.

As an example, consider the data for AUC from a hypothetical crossover study in the following table:

		Reference Article		Test Article	
Animal	Crossover	AUC	LogAUC	AUC	LogAUC

	Sequence				
1	1	518.0	6.25	317.8	5.76
2	1	454.9	6.12	465.0	6.14
3	1	232.8	5.45	548.4	6.31
4	1	311.1	5.74	334.8	5.81
5	2	340.4	5.83	224.7	5.41
6	2	497.7	6.21	249.2	5.52
7	2	652.0	6.48	625.4	6.44
8	2	464.1	6.14	848.7	6.74
	Mean	433.8	6.03	451.7	6.02
	Standard deviation	133.3	0.33	214.3	0.47
	Geometric mean		414.7		410.5

The statistics for AUC will be calculated from the log-transformed data. In this example, *L*, the lower 90% confidence interval calculated on the log scale is -0.395. *U*, the upper 90% confidence interval calculated on the log scale is 0.372. To back-transform these intervals and express them as percentages, we do the following:

Back-transformed lower bound:

$$\left(e^{-0.395}-1\right)\times 100=\left(0.674-1\right)\times 100=\left(-0.326\right)\times 100=-32.6\%$$

Back-transformed upper bound:

$$\left(e^{0.372}-1\right)\times 100=\left(1.451-1\right)\times 100=\left(0.451\right)\times 100=45.1\%$$

Therefore the lower end of the confidence bound lies within -32.6% of the geometric mean of the reference article, while the upper end of the confidence interval lies within 45.1% of the geometric mean of the reference article. If it were determined by CVM that the acceptable confidence bound was 80% to125% of the geometric mean of the reference article in order to demonstrate bioequivalence, then the back-transformed lower bound can be as low as -20% and the back-transformed upper bound can be as high as 25%. In this example, we would determine that the study had not demonstrated an acceptable level of bioequivalence between the test article and the reference article.

A more detailed derivation of these expressions for upper and lower confidence bounds is found in the Appendix.

The width of the confidence interval is determined by the within subject variance (between subject variance for parallel group studies) and the number of subjects in the study. In general, the confidence interval for untransformed data should be 80-120% (the confidence interval should lie within ± 20% of the mean of the reference product). For logarithmically transformed data, the confidence interval is generally 80-125% (the confidence interval should lie within –20% to +25% of the mean of the reference product). The sponsor and CVM should determine the acceptable bounds for confidence limits for the particular drug and formulation during protocol development.

IV. PHARMACOLOGIC END-POINT STUDIES

Where the direct measurement of the rate and extent of absorption of the new animal drug in biological fluids is inappropriate or impractical, the evaluation of a pharmacologic end-point related to the labeled indications for use will be acceptable.

A. General Design Aspects

Typically the design of a pharmacologic end-point study should follow the same general considerations as the blood level studies. However, specifics such as the number of subjects or sampling times will depend on the pharmacologic end-point monitored. The parameters to be measured will also depend upon the pharmacologic end-points and may differ from those used in blood level studies. As with blood level studies, when pharmacologic end-point studies are used to demonstrate bioequivalence, a tissue residue study will also be required in food-producing animals.

B. Statistical Analysis

For parameters which can be measured over time, a time *vs* effect profile is generated, and equivalence is determined with the method of statistical analysis essentially the same as for the blood level bioequivalence study.

For pharmacologic effects for which effect *vs* time curves can not be generated, then alternative procedures for statistical analysis should be discussed with CVM prior to conducting the study.

V. CLINICAL END-POINT STUDIES

If measurement of the drug or its metabolites in blood, biological fluids or tissues is inappropriate or impractical, and there are no appropriate pharmacologic end-points to monitor (*e.g.*, most production drugs and some coccidiostats and anthelmintics), then well-controlled clinical end-point studies are acceptable for the demonstration of bioequivalence.

A. General Design Aspects

Generally, a parallel group design with three treatment groups should be used. The groups should be a placebo (or negative) control, a positive control (reference/pioneer product) and the test (generic) product. The purpose of the placebo (or negative) control is to confirm the sensitivity or validity of the study.

Dosage(s) approved for the pioneer product should be used in the study. Dosage(s) should be selected following consultation with CVM and should reflect consideration for experimental sensitivity and relevance to the common use of the pioneer product.

B. Subject Characteristics and Data Collection

Studies should generally be conducted using the target animal species, with consideration for the sex, class, body weight, age, health status, and feeding and husbandry conditions, as described on the pioneer product labeling. In general, the length of time that the study is conducted should be consistent with the duration of use on the pioneer product labeling.

In general, the response(s) to be measured in a clinical end-point study should be based upon the labeling claims of the pioneer product and selected in consultation with CVM. It may not be necessary to collect data on some overlapping claims (*e.g.*, for a production drug which is added at the same amount per ton of feed for both growth rate and feed efficiency, data from only one of the two responses need be collected).

C. Statistical Analysis

When considering sample size, it is important to note that the pen, not the individual animal, is often the experimental unit.

As with blood level bioequivalence studies, CVM is advocating the use of 90% confidence intervals as the best method for evaluating clinical end-point studies. The bounds for confidence limits (*e.g.*, ± 20% of the improvement over placebo [or negative] control) for the particular drug should be agreed upon with CVM prior to initiation of the study.

The analysis should be used to compare the test product and the reference product. In addition, a traditional hypothesis test should be performed comparing both the test and reference products separately to the placebo (or negative) control. The hypothesis test is conducted to ensure that the study has adequate sensitivity to detect differences when they actually occur. If no significant improvement ($\alpha = .05$) is seen in the parameter (*i.e.*, the mean of the test and the mean of the reference products are each not significantly better than the mean of the placebo [or negative] control), generally, the study will be considered inadequate to evaluate bioequivalence.

Assuming that the test and reference products have been shown to be superior to the placebo (or negative) control, the determination of bioequivalence is based upon the confidence interval of the difference between the two products.

Some clinical end-point studies may not include a placebo (or negative) control for ethical and/or practical considerations. If the placebo is omitted, then the response(s) to the test and reference products should each provide a statistically significant improvement over baseline.

If the results are ordered categorical data (*e.g.*, excellent, good, fair or poor), a non-parametric hypothesis test of no difference between test product and placebo (or negative) control and between the reference product and placebo (or negative) control should be performed. As above, if these tests result in significant differences between the test product and control and the reference product and control, then a non-parametric confidence interval on the difference between the test and reference products is calculated.

Another acceptable approach for categorical data is to calculate the confidence interval on the odds ratio between the test and reference products after showing that the test and reference products are significantly better than the control[11].

VI. HUMAN FOOD SAFETY CONSIDERATIONS

The toxicology and tolerance developed for the pioneer animal drug are applied to generic copies of the drug.

The Panel on Human Food Safety at the 1993 Veterinary Drug Bioequivalence Workshop addressed tissue residue depletion studies for generic animals drugs[1]. The Center has concluded that in addition to a bioequivalence study, a tissue residue depletion study should be conducted for approval of a generic animal drug product in a food-producing species. Two drug products may have the same plasma disposition profile at the concentrations used to assess product bioequivalence, but may have very different tissue disposition kinetics when followed out to the withdrawal time for the pioneer product. Therefore, to show the withdrawal period at which residues of the generic product will be consistent with the tolerance for the pioneer product, a tissue residue depletion study is necessary.

The results of a bioequivalence study or tissue residue depletion study in one animal species can not generally be extrapolated to another species. Possible species differences in drug partitioning or binding in tissues could magnify a small difference in the rate or extent of drug absorbed into a large difference in marker residue concentrations in the target tissue. Therefore, for a pioneer product labeled for more than one food-producing species, a bioequivalence study and a tissue residue depletion study will generally be requested for each major food-producing species on the label.

A traditional withdrawal study, as described in CVM's guidance number 3, "General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals," is considered the best design for collecting data useful for the calculation of a preslaughter withdrawal period for drugs used in food-producing animals. In the traditional withdrawal study, twenty animals are divided into four or five groups of four to five animals each. Groups of animals are slaughtered

at carefully preselected time points following the last administration of the test product and the edible tissues are collected for residue analysis. A statistical tolerance limit approach is used to determine when, with 95% confidence, 99% of treated animals would have tissue residues below the codified limits.

For purposes of calculating a withdrawal period for a generic animal drug, only the generic product would be tested (i.e., not the pioneer product), and only the marker residue in the target tissue would be analyzed.

Other study designs will be considered on a case-by-case basis. Sponsors are encouraged to submit the proposed tissue residue depletion protocol for CVM concurrence before proceeding with the withdrawal study.

The generic animal drug will be assigned the withdrawal time supported by the residue depletion data, or the withdrawal time currently assigned to the pioneer product, whichever is the longer.

The generic animal drug sponsor may request a shorter withdrawal period for the generic product by supplementing the ANADA and providing tissue residue data necessary to support the shorter withdrawal period request. Such a supplement will be reviewed under the agency's policy for Category II supplements. For a Category II supplement, a reevaluation of the safety (or effectiveness) data in the parent application (i.e., the pioneer NADA) may be required (21 CFR 514.106 (b) (2)). The Center will ordinarily approve a request for a shorter withdrawal period when the residue data are adequate and when no other human food safety concerns for the drug are evident.

Under 21 CFR 514.1(b)(7), applications are required to include a description of practicable methods for determining the quantity, if any, of the new animal drug in or on food, and any substance formed in or on food because of its use, and the proposed tolerance or withdrawal period or other use restrictions to ensure that the proposed use of the drug will be safe. For certain drug products, a tissue residue depletion study is not needed to ensure that residues of the test product will be consistent with the codified drug tolerance at the withdrawal time assigned to the reference product. These include but may not be limited to products for which a waiver of in vivo bioequivalence testing is granted, and products for which the assay method used in the blood level bioequivalence study is sensitive enough to measure blood levels of the drug for the entire withdrawal period assigned to the reference product. Other requests for waiver of the tissue residue study will be considered on a case-by-case basis.

CVM will not request that the assay methodology used to determine the withdrawal period for the generic product be more rigorous than the approved methodology used to determine the existing withdrawal period for the pioneer product. If an analytical method other than the approved method of analysis is used, the generic sponsor should provide data comparing the alternate method to the approved method.

APPENDIX: Confidence Bounds on the Logarithm Scale

We want to develop an expression for the confidence bound of the difference between the pharmacokinetic parameter for the test treatment and the reference treatment, expressed as a percentage of the reference treatment. This bound is derived from the 90% confidence interval of the difference between the mean of the test treatment and the mean of the reference treatment. This appendix addresses the case when the data analysis used to calculate the 90% confidence interval has been done with the natural log of the pharmacokinetic parameter as the dependent variable.

For purposes of this illustration we will use Area Under the Curve (AUC) as the pharmacokinetic parameter.

Notation and Distributional Assumptions:

Area under the Curve for Reference Treatment AUC_R (1)

Area under the Curve for Test Treatment AUC_T (2)

Natural log of AUC $LnAUC$ (3)

$LnAUC_T \sim N(\mu_T, \sigma^2)$ and $LnAUC_R \sim N(\mu_R, \sigma^2)$ (4)

$\overline{LnAUC}_T = \sum_{i=1}^{n} \frac{LnAUC_{iT}}{n}$ which estimates μ_T, similarly for μ_R (5)

$e^{(\overline{LnAUC}_T)}$ is the geometric mean of AUC_T to be denoted by $\dot{\mu}_T$, similarly for $\dot{\mu}_R$ (6)

Calculation of the Confidence Interval:

The 90% Confidence Interval of $(\mu_T - \mu_R)$ is denoted by (L, U) and is calculated from (7)

$$L = \left(\overline{Ln}AUC_T - \overline{Ln}AUC_R\right) - t_{n_A + n_B - 2;.05}\, s\sqrt{\frac{1}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}$$

$$U = \left(\overline{Ln}AUC_T - \overline{Ln}AUC_R\right) + t_{n_A + n_B - 2;.05}\, s\sqrt{\frac{1}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}$$

Manipulating This Expression Gives:

$$L < \mu_T - \mu_R < U \quad (8)$$

$$e^L < e^{\mu_T - \mu_R} < e^U \quad (9)$$

$$e^L < \frac{\mu_T}{\mu_R} < e^U \quad (10)$$

$$e^L - 1 < \frac{\mu_T}{\mu_R} - 1 < e^U - 1 \quad (11)$$

$$e^L - 1 < \frac{\mu_T - \mu_R}{\mu_R} < e^U - 1 \quad (12)$$

Expressed As A Percentage:

$$\left(e^L - 1\right) \times 100 < \left(\frac{\mu_T - \mu_R}{\mu_R}\right) \times 100 < \left(e^U - 1\right) \times 100 \quad (13)$$

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